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(FILE 'CAPLUS' ENTERED AT 08:24:38 ON 11 MAY 2001)
L1 339173 SEA FILE=CAPLUS ABB=ON PLU=ON AUTOTRANSPORTER OR AUTO TRANSPORTER OR ENTEROBACTER? OR BACTERI##
L2 15 SEA FILE=CAPLUS ABB=ON PLU=ON L1 AND AIDA

L2 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:60350 CAPLUS
TITLE: The **AIDA autotransporter** system is associated with F18 and Stx2e in Escherichia coli isolates from pigs diagnosed with edema disease and postweaning diarrhea
AUTHOR(S): Niewerth, Ulla; Frey, Andreas; Voss, Thomas; Le Bouguenec, Chantal; Baljer, Georg; Franke, Sylvia; Schmidt, M. Alexander
CORPORATE SOURCE: Institut fur Infektiologie, Zentrum fur Molekularbiologie der Entzundung, Westfalische Wilhelms-Universitat, Munster, D-48149, Germany
SOURCE: Clin. Diagn. Lab. Immunol. (2001), 8(1), 143-149
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pathogenic Escherichia coli strains are known to cause edema disease (ED) and postweaning diarrhea (PWD) in piglets. Although the exact mechanisms of pathogenicity that lead to ED-PWD remain to be elucidated, E. coli-borne Shiga-like toxin and adhesion-mediating virulence factors such as F18 adhesin or F4 fimbriae are believed to play a central role in ED-PWD. In light of these observations we investigated whether another E. coli adhesin, the plasmid-encoded **AIDA** (adhesin involved in diffuse adherence) might also be present in ED-PWD-causing E. coli isolates. For rapid screening for the **AIDA** system in large nos. of isolates, a multiplex PCR method along with a duplex Western blot procedure was developed. When screening 104 strains obtained from pigs with or without ED-PWD, we obsd. a high prevalence of the **AIDA** operon in porcine E. coli isolates, with over 25% of all strains being **AIDA** pos., and we could demonstrate a significant assocn. of the intact **AIDA** gene (orfB) with ED-PWD, while defects in orfB were assocd. with the absence of disease. Although our data hint toward a contribution of **AIDA** to ED-PWD, further studies will be necessary since the presence of the **AIDA** genes was also assocd. with the presence of the Shiga-like toxin and F18 adhesin genes, two reported virulence factors for ED-PWD.
REFERENCE COUNT: 31
REFERENCE(S): (1) Benz, I; Infect Immun 1989, V57, P1506 CAPLUS
(2) Benz, I; Infect Immun 1992, V60, P13 CAPLUS
(3) Benz, I; Mol Microbiol 1992, V6, P1539

Searcher : Shears 308-4994

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CAPLUS
(7) Engler-Blum, G; Anal Biochem 1993, V210,
P235 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:446280 CAPLUS
DOCUMENT NUMBER: 133:330143
TITLE: Autodisplay: functional display of active .beta.-lactamase on the surface of Escherichia coli by the **AIDA**-I **autotransporter**
AUTHOR(S): Lattemann, Claus T.; Maurer, Jochen; Gerland, Elke; Meyer, Thomas F.
CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut fur Biologie, Tubingen, D-72076, Germany
SOURCE: J. Bacteriol. (2000), 182(13), 3726-3733
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Members of the protein family of IgA1 protease-like **autotransporters** comprise multidomain precursors consisting of a C-terminal **autotransporter** domain that promotes the translocation of N-terminally attached passenger domains across the cell envelopes of Gram-neg. **bacteria**. Several **autotransporter** domains have recently been shown to efficiently promote the export of heterologous passenger domains, opening up an effective tool for surface display of heterologous proteins. Here we report on the **autotransporter** domain of the Escherichia coli adhesin involved in diffuse adherence (**AIDA**-I), which was genetically fused to the C terminus of the periplasmic enzyme .beta.-lactamase, leading to efficient expression of the fusion protein in E. coli. The .beta.-lactamase moiety of the fusion protein was presented on the **bacterial** surface in a stable manner, and the surface-located .beta.-lactamase was shown to be enzymically active. Enzymic activity was completely removed by protease treatment, indicating that surface display of .beta.-lactamase was almost quant. The periplasmic domain of the outer membrane protein OmpA was not affected by externally added proteases, demonstrating that the outer membranes of E. coli cells expressing the .beta.-lactamase **AIDA**-I fusion protein remained physiol. intact.
REFERENCE COUNT: 39
REFERENCE(S): (1) Bardwell, J; Cell 1991, V67, P581 CAPLUS

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CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:189528 CAPLUS
DOCUMENT NUMBER: 133:16059
TITLE: Cell surface presentation of recombinant (poly-) peptides including functional T-cell epitopes by the **AIDA autotransporter** system
AUTHOR(S): Konieczny, M. P. J.; Suhr, M.; Noll, A.; Autenrieth, I. B.; Schmidt, M. Alexander
CORPORATE SOURCE: Institut fur Infektiologie-Zentrum fur Molekularbiologie der Entzundung (ZMBE), Westfalische Wilhelms-Universitat Munster, Munster, 48149, Germany
SOURCE: FEMS Immunol. Med. Microbiol. (2000), 27(4), 321-332
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB For the efficient surface presentation and release of virulence factors esp. pathogenic Gram-neg. **bacteria** have developed several distinct secretion mechanisms. An increasing no. of pathogens in various species employs a mechanism denoted the '**autotransporter**' pathway. This pathway is characterized by an outer membrane translocator module representing the C-terminal domain of the transported protein itself. An intriguing potential application of such systems involves the transport and surface expression of recombinant proteins or peptides, like e.g. the presentation of antigens for the generation of live oral vectors as vaccine carriers. Here the authors report on the incorporation of heterologous (poly-) peptides in permissive sites of the translocator module of the adhesin-involved-in-diffuse-adherence (**AIDA**) **autotransporter** system. They demonstrate the presentation of the B subunit of the heat labile enterotoxin of Escherichia coli (LTB) as well as of functional T-cell epitopes of Yersinia enterocolitica heat-shock protein 60 (Y-hsp60) on the surface of E. coli.

REFERENCE COUNT: 33

REFERENCE(S): (1) Achtman, M; Infect Immun 1983, V39, P315 CAPLUS
(3) Benz, I; Infect Immun 1989, V57, P1506 CAPLUS
(4) Benz, I; Infect Immun 1992, V60, P13 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:750885 CAPLUS
DOCUMENT NUMBER: 132:60602
TITLE: Characterization of the essential transport function of the **AIDA**-I **autotransporter** and evidence supporting structural predictions
AUTHOR(S): Maurer, Jochen; Jose, Joachim; Meyer, Thomas F.
CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut fur Biologie, Tubingen, D-72076, Germany
SOURCE: J. Bacteriol. (1999), 181(22), 7014-7020
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The current model for autodisplay suggests a mechanism that allows a passenger protein to be translocated across the outer membrane by coordinate action of a C-terminal .beta.-barrel and its preceding linking region. The passenger protein, linker, and .beta.-barrel are together termed the **autotransporter**, while the linker and .beta.-barrel are here referred to as the translocation unit (TU). We characterized the minimal TU necessary for autodisplay with the adhesin-involved-in-diffuse-adherence (**AIDA**-I) **autotransporter**. The assumed .beta.-barrel structure at the C terminus of the **AIDA**-I **autotransporter** was studied by constructing a set of seven **AIDA**-I-cholera toxin B subunit fusion proteins contg. various portions of **AIDA**-I. Surface exposure of the cholera toxin B moiety was assessed by dot blot expts. and trypsin accessibility of the chimeric proteins expressed in Escherichia coli JK321 or UT5600. Export of cholera toxin B strictly depended on a complete predicted .beta.-barrel region. The abs. necessity for export of a linking region and its influence on expression as an integral part of the TU was also demonstrated. The different electrophoretic mobilities of native and denatured chimeras indicated that the proposed .beta.-barrel resides within the C-terminal 312 amino acids of

AIDA-I. Together these data provide evidence for the predicted .beta.-barrel structure and support our formerly proposed model of membrane topol. of the **AIDA**-I **autotransporter**.

REFERENCE COUNT: 54

REFERENCE(S):

(1) Anderson, D; Science 1997, V278, P1140 CAPLUS
(2) Baneyx, F; Ann NY Acad Sci 1992, V665, P301 CAPLUS
(3) Baneyx, F; Appl Microbiol Biotechnol 1991, V36, P14 CAPLUS
(4) Baneyx, F; J Bacteriol 1990, V172, P491 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:486643 CAPLUS

DOCUMENT NUMBER: 131:240143

TITLE: Identification of a glycoprotein produced by enterotoxigenic Escherichia coli

AUTHOR(S): Lindenthal, Christoph; Elsinghorst, Eric A.

CORPORATE SOURCE: Department of Molecular Biosciences, University of Kansas, Lawrence, KS, 66045-2106, USA

SOURCE: Infect. Immun. (1999), 67(8), 4084-4091
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enterotoxigenic Escherichia coli (ETEC) strain H10407 is capable of invading epithelial cell lines derived from the human ileocecum and colon in vitro. Two sep. chromosomally encoded invasion loci (tia and tib) have been cloned from this strain. These loci direct nonadherent and noninvasive lab. strains of E. coli to adhere to and invade cultured human intestinal epithelial cells. The tib locus directs the synthesis of TibA, a 104-kDa outer membrane protein that is directly correlated with the adherence and invasion phenotypes. TibA is synthesized as a 100-kDa precursor (preTibA) that must be modified for biol. activity. Outer membranes of recombinant E. coli expressing TibA or preTibA were sepd. by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted to nitrocellulose. The presence of glycoproteins was detected by oxidization of carbohydrates with periodate and labeling with hydrazide-conjugated digoxigenin. Only TibA could be detected as a glycoprotein. Complementation expts. with tib deletion mutants of ETEC strain H10407 demonstrate that the TibA glycoprotein is expressed in H10407, that the entire tib locus is required for TibA

synthesis, and that TibA is the only glycoprotein produced by H10407. Protease treatment of intact H10407 cells removes the carbohydrates on TibA, suggesting that they are surface exposed. TibA shows homol. with **AIDA**-I from diffuse-adhering E. coli and with pertactin precursor from Bordetella pertussis. Both pertactin and **AIDA**-I are members of the **autotransporter** family of outer membrane proteins and are afimbrial adhesins that play an important role in the virulence of these organisms. Anal. of the predicted TibA amino acid sequence indicates that TibA is also an **autotransporter**. Anal. of the tib locus DNA sequence revealed an open reading frame with similarity to RfaQ, a glycosyltransferase. The product of this tib locus open reading frame is proposed to be responsible for TibA modification. These results suggest that TibA glycoprotein acts as an adhesin that may participate in the disease process.

REFERENCE COUNT: 47

REFERENCE(S):
(1) Benz, I; Mol Microbiol 1992, V6, P1539 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:229125 CAPLUS
DOCUMENT NUMBER: 128:279562
TITLE: A transport and secretion system for the presentation of foreign proteins on the surface of Gram-negative **bacteria**
INVENTOR(S): Schmidt, M. Alexander; Suhr, Martin; Benz, Inga
PATENT ASSIGNEE(S): Schmidt, M. Alexander, Germany
SOURCE: Ger. Offen., 6 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19641158	A1	19980409	DE 1996-19641158	19961007

AB A system that efficiently transports foreign proteins to the cell surface of Gram-neg. **bacteria** where they may be displayed or secreted into the medium uses fusion proteins with the C-terminal

440-amino acid fragment of the adhesin **AIDA**-1 to direct proteins to the cell surface. The protein can then be anchored on the cell surface, or the domains of the fusion protein can be linked by a peptide that is cleaved by a cell membrane proteinase such as ompT. Suitable expression constructs are also described.

L2 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:736605 CAPLUS
DOCUMENT NUMBER: 128:45638
TITLE: Hierarchical autoinduction in Ralstonia solanacearum: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester
AUTHOR(S): Flavier, Albert B.; Ganova-Raeva, Lilia M.; Schell, Mark A.; Denny, Timothy P.
CORPORATE SOURCE: Departments of Plant Pathology, University of Georgia, Athens, GA, 30602, USA
SOURCE: J. Bacteriol. (1997), 179(22), 7089-7097
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Bacteria** employ autoinduction systems to sense the onset of appropriate cell d. for expression of developmental genes. In many gram-neg. **bacteria**, autoinduction involves the prodn. of and response to diffusible acylated-homoserine lactones (acyl-HSLs) and is mediated by members of the LuxR and LuxI families. Ralstonia (Pseudomonas) solanacearum, a phytopathogenic **bacterium** that appears to autoregulate its virulence genes, produces compds. that promote expression of several heterologous acyl-HSL-responsive reporter gene constructs. High-pressure liq. chromatog. of highly concd. Et acetate exts. revealed that culture supernatants of strain AW1 contained two compds. with retention times similar to N-hexanoyl- and N-octanoyl-HSL. To investigate the role of these acyl-HSLs in R. solanacearum virulence gene expression, transposon mutants that were deficient for inducing an acyl-HSL-responsive reporter in Agrobacterium tumefaciens were generated. Three loci involved in normal acyl-HSL prodn. were identified, one of which was shown to contain the divergently transcribed solR and solI genes, the luxR and luxI homologs, resp. A 4.1-kb fragment contg. solR and solI enabled all of the mutants (regardless of the locus inactivated) and a naturally acyl-HSL-defective strain of R. solanacearum to produce acyl-HSLs. Inactivation of solI abolished prodn. of all detectable acyl-HSLs but affected neither the expression of virulence genes in culture nor the ability to wilt tomato plants. AW1 has a functional autoinduction system, because (i) expression of solI required SolR

and acyl-HSL and (ii) expression of a gene linked to solR and solI, designated **aidA**, was acyl-HSL dependent. Because **AidA** has no homologs in the protein databases, its discovery provided no clues as to the role of acyl-HSLs in R. solanacearum gene regulation. However, expression of solR and solI required the global LysR-type virulence regulator PhcA, and both solR and solI exhibited a cell d.-assocd. pattern of expression similar to other PhcA-regulated genes. The acyl-HSL-dependent autoinduction system in R. solanacearum is part of a more complex autoregulatory hierarchy, since the transcriptional activity of PhcA is itself controlled by a novel autoregulatory system that responds to 3-hydroxypalmitic acid Me ester.

L2 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:640781 CAPLUS
DOCUMENT NUMBER: 127:315572
TITLE: Recombinant protein fusion products presentation on **bacteria** cell surface and release by proteinase
INVENTOR(S): Maurer, Jochen; Jose, Joachim; Meyer, Thomas F.
PATENT ASSIGNEE(S): Max-Planck-Gesellschaft zur Forderung der Wissenschaften E.V., Berlin, Germany; Maurer, Jochen; Jose, Joachim; Meyer, Thomas F.
SOURCE: PCT Int. Appl., 84 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9735022	A1	19970925	WO 1996-EP1130	19960315
W: AU, CA, CN, JP, KR, NZ, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2248754	AA	19970925	CA 1996-2248754	19960315
AU 9651097	A1	19971010	AU 1996-51097	19960315
AU 714389	B2	19991223		
EP 886678	A1	19981230	EP 1996-907487	19960315
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1216065	A	19990505	CN 1996-180254	19960315
JP 2000504928	T2	20000425	JP 1997-519186	19960315

PRIORITY APPLN. INFO.: WO 1996-EP1130 A 19960315

AB The present invention relates to vectors, host-vector combinations and processes for producing stable fusion proteins consisting of a carrier protein and a passenger protein. Expression of the fusion

protein results in exposure of the passenger domains on the surface of **bacterial** cells, in particular Escherichia coli. If necessary, the passenger domains can be released into the medium by proteases, e.g. by selected host factors such as OmpT.

L2 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:88914 CAPLUS
DOCUMENT NUMBER: 126:155032
TITLE: Autodisplay: one-component system for efficient surface display and release of soluble recombinant proteins from Escherichia coli
AUTHOR(S): Mauerer, Jochen; Jose, Joachim; Meyer, Thomas F.
CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut Biol., Berlin, 10117, Germany
SOURCE: J. Bacteriol. (1997), 179(3), 794-804
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The IgA protease family of secreted proteins are derived from self-translocating polyprotein precursors which contain C-terminal domains promoting the translocation of the N-terminally attached passenger domains across gram-neg. **bacterial** outer membranes. Computer predictions identified the C-terminal domain of the Escherichia coli adhesin involved in diffuse adherence (**AIDA**-I) as a member of the **autotransporter** family. A model of the .beta.-barrel structure, proposed to be responsible for outer membrane translocation, served as a basis for the construction of fusion proteins contg. heterologous passengers. **Autotransporter**-mediated surface display (autodisplay) was investigated for the cholera toxin B subunit and the peptide antigen tag PEYFK. Up to 5% of total cellular protein was detectable in the outer membrane as passenger **autotransporter** fusion protein synthesized under control of the constitutive PTK promoter. Efficient presentation of the passenger domains was demonstrated in the outer membrane protease T-deficient (ompT) strain E. coli UT5600 and the ompT dsbA double mutant JK321. Surface exposure was ascertained by ELISA, immunofluorescence microscopy, and immunogold electron microscopy using antisera specific for the passenger domains. In strain UT2300 (ompT+), the passenger domains were released from the cell surface by the OmpT protease at a novel specific cleavage site, R .dwnarw. V. Autodisplay represents a useful tool for future protein translocation studies with interesting biotechnol. possibilities.

L2 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:726933 CAPLUS

DOCUMENT NUMBER: 126:57127
TITLE: Phase-variable outer membrane proteins in Escherichia coli
AUTHOR(S): Owen, Peter; Meehan, Mary; de Loughry-Doherty, Helen; Henderson, Ian
CORPORATE SOURCE: Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College Dublin, Dublin, 2, Ire.
SOURCE: FEMS Immunol. Med. Microbiol. (1996), 16(2), 63-76
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 70 refs. Escherichia coli contains at least two phase-variable proteins in its outer membrane. One, termed antigen 43 (Ag43), is the product of the metastable flu gene located at min 43.6 on the E. coli chromosome and is responsible for colony form variation and for autoaggregation in liq. media. Ag43 is composed of two proteinaceous subunits, .alpha.43 and .beta.43, in 1:1 stoichiometry. The .alpha.43 (apparent Mr 60,000) is surface expressed, extends beyond the O-side chains of smooth lipopolysaccharide and is bound to the cell surface through an interaction with .beta.43 (apparent Mr 53,000), itself an integral, heat-modifiable, outer membrane protein. The .alpha.43 shows limited N-terminal sequence homol. with certain **enterobacterial** adhesins and notable sequence homol. with **AIDA**-1, an adhesin of diffuse-adhering E. coli. In addn., .alpha.43 contains an RGD motif and a consensus sequence for an (autoproteolytic) aspartyl protease active site. Expression of Ag43 is subject to reversible phase variation. In liq. minimal medium, the rates of variation from Ag43+ to Ag43- states and from Ag43- to Ag43+ states are .apprxeq.2.2.times.10-3 and .apprxeq.1.times.10-3, resp. Phase switching of Ag43 is regulated by DNA methylation (deoxyadenosine methylase (dam) mutants being 'locked OFF') and by OxyR (oxyR mutants being 'locked ON'). It is proposed that OxyR acts as a repressor of Ag43 transcription by binding to unmethylated GATC sites in the regulatory region of the gene. In some strains, Ag43 may also undergo antigenic variation. A 94 kDa immunocrossreactive outer membrane protein, showing similar rates of phase variation, has addnl. been detected for some enteropathogenic and uropathogenic strains of E. coli. This 94 kDa protein can be proteolytically cleaved in situ with trypsin to yield two membrane-bound products with Mrs and properties similar to those of .alpha.43 and .beta.43. Ag43 may represent one of a family of antigenically related high-Mr adhesins which are synthesized as polyprotein precursors. Some members may be processed and presented on the cell surface as bipartite protein complexes (as Ag43).

Others can remain uncleaved.

L2 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:629212 CAPLUS
DOCUMENT NUMBER: 125:270137
TITLE: Processing of the **AIDA**-I precursor: removal of AIDAc and evidence for the outer membrane anchoring as a .beta.-barrel structure
AUTHOR(S): Suhr, Martin; Benz, Inga; Schmidt, M. Alexander
CORPORATE SOURCE: Inst. Infektiologie, Zentrum Molekularbiol. Entzuendung (ZMBE), Zentrum Molekularbiol., Muenster, D-48149, Germany
SOURCE: Mol. Microbiol. (1996), 22(1), 31-42
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **AIDA**-I adhesin known to be responsible for the diffuse adherence (DA) phenotype of the diarrheagenic Escherichia coli (DAEC) strain 2787 has been shown previously to be synthesized as a precursor protein and to undergo addnl. C-terminal processing. Here, the C-terminal processing of the **AIDA**-I precursor and the outer membrane topol. of the cleaved C-terminal fragment, AIDAc, were investigated. By isolation of the cleaved AIDAc fragment and N-terminal sequencing, the C-terminal cleavage site was identified between Ser-846 and Ala-847, thereby indicating a mol. mass of 47.5 kDa for AIDAc. The correct processing to **AIDA** -I and AIDAc in OmpT, OmpP and DegP protease-deficient E. coli strains as well as in avirulent salmonellae and shigellae points to an autocatalytic cleavage mechanism. The cleaved AIDAc was localized in the outer membrane. A leader sequence-AIDAc fusion was efficiently routed to the outer membrane. Anal. by protease digestion, secondary structure prediction and modeling, by comparison with structurally related **bacterial** proteins like the IgA1 protease from Neisseria, the vacuolating toxin from Helicobacter pylori, and the VirG protein of Shigella flexneri, strongly indicates that AIDAc is present in the outer membrane as a .beta.-barrel structure.

L2 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:719729 CAPLUS
DOCUMENT NUMBER: 124:22809
TITLE: Whole-genome random sequencing and assembly of Haemophilus influenzae Rd
AUTHOR(S): Fleischmann, Robert D.; Adams, Mark D.; White, Owen; Clayton, Rebecca A.; Kirkness, Ewen F.; Kerlavage, Anthony R.; Bult, Carol J.; Tomb, Jean-Francois; Dougherty, Brian A.; et al.
CORPORATE SOURCE: Inst. Genomic Res., Gaithersburg, MD, 20878, USA

SOURCE: Science (Washington, D. C.) (1995), 269(5223), 496-8, 507-12
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An approach for genome anal. based on sequencing and assembly of unselected pieces of DNA from the whole chromosome has been applied to obtain the complete nucleotide sequence (1,830,137 base pairs) of the genome from the **bacterium** Haemophilus influenzae Rd. This approach eliminates the need for initial mapping efforts and is therefore applicable to the vast array of microbial species for which genomes maps are unavailable. The H. influenzae Rd genome sequence (Genome Sequence DataBase accession no. L42023) represents the only complete genome sequence from a free-living organism.

L2 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:290460 CAPLUS
DOCUMENT NUMBER: 122:76148
TITLE: Survey of clinical isolates of diarrheogenic Escherichia coli: diffusely adhering E. coli strains with multiple adhesive factors
AUTHOR(S): Jallat, C.; Darfeuille-Michaud, A.; Rich, C.; Joly, B.
CORPORATE SOURCE: Laboratorie de Bacteriologie, Faculte de Pharmacie, Clermont-Ferrand, 63001, Fr.
SOURCE: Res. Microbiol. (1994), 145(8), 621-32
CODEN: RMCREW; ISSN: 0923-2508
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A total of 335 Escherichia coli strains were isolated from sporadic cases of aq. diarrhea in patients hospitalized in Clermont-Ferrand, France, during 1991 and 1992. Many of these strains belonged to the diffusely adhering E. coli (DAEC) group, since 51 of them (15.2%) hybridized with the daaC probe corresponding to the accessory gene of the F1845 adhesin and 13 (3.9%) with the **AIDA**-I (adhesin involved in diffuse adhesion-I) structural gene. The other pathogenic E. coli groups were weakly represented: 0.6% (2 strains) of enterotoxigenic E. coli (ETEC), 0.6% (2 strains) of enterohemorrhagic E. coli (EHEC) and 3.9% (13 strains) of enteroaggregative E. coli (EAggEC). Neither enteropathogenic E. coli (EPEC) nor enteroinvasive E. coli (EIEC) were isolated in our study period. Among the DAEC strains studied, we described two major surface proteins of 16 and 29 kDa. We showed that the 16-kDa protein (CF16K) was involved in adhesion in vitro to Caco-2 and HEp-2 cells. Pretreatment of **bacteria** with anti-CF16K serum or of Caco-2 cells with purified CF16K greatly decreased the adhesion of the E. coli CF1085 strain producing the CF16K protein to both cell types. The CF16K adhesive factor was found in 9.5% (33

strains) of the 335 E. coli strains studied by colony immunoblot assays with anti-CF16K serum. Twelve strains producing CF16K hybridized with the daaC probe, indicating that the CF16K is not related to the Dr family adhesins which recognized the Dr blood group antigen as receptor. The 29-kDa protein, isolated from 9 strains out of the 335 studied (5.1%), was identified as the CS31A antigen by Western blot assay using anti-CS31A serum and by hybridization expts. with a CS31A DNA probe. This antigen is routinely obsd. in septicemic or enterotoxigenic bovine E. coli strains. We showed that a single diarrheogenic E. coli strain could harbor at least two adhesive factors, since 36% of CF16K E. coli strain producers and 68.4% of CS31A E. coli strain producers hybridized with the daaC DNA probe.

L2 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:596937 CAPLUS
DOCUMENT NUMBER: 119:196937
TITLE: Association between the effacing (eae) gene and the shiga-like toxin-encoding genes in Escherichia coli isolates from cattle
AUTHOR(S): Mainil, Jacques G.; Jacquemin, Etienne R.; Kaeckenbeeck, Albert E.; Pohl, Pierre H.
CORPORATE SOURCE: Fac. Vet. Med., Univ. Liege, Liege, B-4000, Belg.
SOURCE: Am. J. Vet. Res. (1993), 54(7), 1064-8
CODEN: AJVRAH; ISSN: 0002-9645
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two hundred ninety-six Escherichia coli isolates from feces or intestines of calves with diarrhea were hybridized with 7 gene probes. One probe (the eae probe) was derived from the eae gene coding for a protein involved in the effacement of the enterocyte microvilli by the group of **bacteria** called attaching and effacing E coli (AEEC), and 2 probes were derived from genes coding for the Shiga-like toxins (SLT) I and II produced by the verocytotoxic E coli (VTEC). The other 4 probes were derived from DNA sequences assocd. with the adhesive properties of enteroadherent E coli (EAEC) to cultured cells (the EAF probe for the localized adherence pattern, probes F1845 and **AIDA**-1 for the diffuse adherence pattern, and the Agg probe for the aggregative adherence pattern). Hybridization results for the eae probe were in agreement, for all but 1 of the 8 isolates, with previously published phenotypic results of microvilli effacement. The latter was previously reported as effacing the microvilli of calf enterocytes, but was eae probe-neg. Two classes of isolates hybridized with the eae probe. Members of a first class (60 isolates) addnl. produced a pos. signal with 1 or both of the SLT probes (VTEC-AEEC isolates). Isolates hybridizing with the eae and

the SLT1 probes were the most frequent: 56 isolates (ie, 93% of all VTEC-AEEC). Members of the second class (10 isolates) failed to hybridize with either SLT probe (non-VTEC-AEEC isolates). Most isolates of these 2 classes belong to only 4 serogroups: O5, O26, O111, and O118. In addn. to these 2 AEEC classes, a VTEC class (20 isolates) was obsd. Such isolates were pos. with 1 or both SLT probes, but were neg. with the eae probe. All but 1 isolate belonged to serogroups not found among the AEEC isolates. Only 7 of all AEEC and VTEC isolates were pos. with the EAF, the F1845, or the **AIDA**-1 probe, and none were pos. with the Agg probe. On the other hand, 32 non-VTEC, non-AEEC isolates were pos. with the F1845 probe only, 2 were pos. with the EAF probe only, and 1 was pos. with the **AIDA**-1 probe only, thus constituting a possible class of EAEC isolates from cattle. The eae gene and the gene coding for the SLT1 are, thus assocd. in most AEEC isolates from cattle. The isolates with other hybridization results (VTEC and EAEC isolates) need more work to be clearly defined.

L2 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:81336 CAPLUS
DOCUMENT NUMBER: 116:81336
TITLE: Isolation and serologic characterization of **AIDA**-I, the adhesin mediating the diffuse adherence phenotype of the diarrhea-associated Escherichia coli strain 2787 (O126:H27)
AUTHOR(S): Benz, Inga; Schmidt, M. Alexander
CORPORATE SOURCE: Zent. Mol. Biol., Univ. Heidelberg, Heidelberg, D-6900, Germany
SOURCE: Infect. Immun. (1992), 60(1), 13-18
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The adherence of diarrhea-assocd. E. coli to the small-bowel mucosa is an important step in the pathogenesis of diarrheal diseases. In tissue culture systems, diarrhea-assocd. strains show 3 patterns of adherence: localized adherence, diffuse adherence (DA), and the recently described aggregative adherence. To study the mol. basis of the DA phenotype, the authors investigated the diarrhea-assocd. DA strain 2787 (O126:H27), isolated from a case of infantile diarrhea. The DA phenotype is mediated by a 6.0-kb DNA fragment derived from a 100-kb plasmid harbored by the wild-type strain. This fragment codes for a 100-kDa protein which can be released from the **bacterial** cell into the supernatant by mild heat treatment. Recombinant DA+ strains as well as the isolated 100-kDa protein were used to engender specific antisera in rabbits. As demonstrated by Western blotting (immunoblotting), the antibodies engendered by the recombinant DA+ strain recognized a 100-kDa

protein in the wild-type strain 2787 and in all recombinant strains showing DA. Immunogold electron microscopy localized the 100-kDa protein to the **bacterial** cell surface. Serol. related proteins of similar size were detected by Western blotting in other DA+ diarrhea-assocd. strains belonging to enteropathogenic E. coli serotypes. The 100-kDa protein denoted **AIDA**-I (adhesion involved in diffuse adherence) binds in a saturable fashion to HeLa cells. **AIDA**-I-specific IgG antibodies and to a greater extent, Fab fragments derived thereof, inhibited **bacterial** attachment to HeLa cells. Thus, the 100-kDa protein is the adhesion mediating the DA phenotype of these diarrhea-assocd. strains and is representative of a group of serol. related proteins in other DA+ strains.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 08:31:46 ON 11 MAY 2001)

L3 104 S L2
L4 7 S L3 AND VECTOR
L5 2 DUP REM L4 (5 DUPLICATES REMOVED)

L5 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000193544 MEDLINE
DOCUMENT NUMBER: 20193544 PubMed ID: 10727888
TITLE: Cell surface presentation of recombinant (poly-) peptides including functional T-cell epitopes by the **AIDA autotransporter** system.
AUTHOR: Konieczny M P; Suhr M; Noll A; Autenrieth I B; Alexander Schmidt M
CORPORATE SOURCE: Institut fur Infektiologie-Zentrum fur Molekularbiologie der Entzundung (ZMBE), Westfalische Wilhelms-Universitat Munster, Von-Esmarch-Str. 56, 48149, Munster, Germany.
SOURCE: FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, (2000 Apr) 27 (4) 321-32.
Journal code: BP1; 9315554. ISSN: 0928-8244.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000511

AB For the efficient surface presentation and release of virulence factors especially pathogenic Gram-negative **bacteria** have developed several distinct secretion mechanisms. An increasing number of pathogens in various species employs a mechanism denoted the '**autotransporter**' pathway. This pathway is

characterised by an outer membrane translocator module representing the C-terminal domain of the transported protein itself. An intriguing potential application of such systems involves the transport and surface expression of recombinant proteins or peptides, like e.g. the presentation of antigens for the generation of live oral **vectors** as vaccine carriers. Here we report on the incorporation of heterologous (poly-) peptides in permissive sites of the translocator module of the adhesin-involved-in-diffuse-adherence (**AIDA**) **autotransporter** system. We demonstrate the presentation of the B subunit of the heat labile enterotoxin of Escherichia coli (LTB) as well as of functional T-cell epitopes of Yersinia enterocolitica heat-shock protein 60 (Y-hsp60) on the surface of E. coli.

L5 ANSWER 2 OF 2 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 89212899 MEDLINE
DOCUMENT NUMBER: 89212899 PubMed ID: 2565291
TITLE: Cloning and expression of an adhesin (**AIDA**-I) involved in diffuse adherence of enteropathogenic Escherichia coli.
AUTHOR: Benz I; Schmidt M A
CORPORATE SOURCE: Zentrum fur Molekulare Biologie Heidelberg, Universitat Heidelberg, Federal Republic of Germany.
SOURCE: INFECTION AND IMMUNITY, (1989 May) 57 (5) 1506-11. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198906
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19950206
Entered Medline: 19890601

AB The adherence of enteropathogenic Escherichia coli (EPEC) to the small bowel mucosa is an important step in the pathogenesis of diarrheal diseases. It has been shown that many EPEC strains adhere to HEp-2 and especially HeLa cells in characteristic patterns termed localized adherence (LA) and diffuse adherence (DA). A plasmid-derived DNA fragment encoding a factor specific for LA hybridized only to EPEC strains expressing LA, which demonstrated that LA and DA are mediated by two genetically distinct adhesins. EPEC strain 2787 (O127:H27), isolated from a case of infantile diarrhea, exhibited three major properties: (i) it showed DA to HeLa cells, (ii) it carried two large (ca. 100-kilobase [kb]) plasmids and one small plasmid of about 3 kb, and (iii) no fimbriae could be detected by electron microscopy in organisms grown on agar plates or in liquid cultures. Whole isolated plasmid DNA was partially digested with EcoRI and cloned into the **vector** pBR322. One

recombinant clone (pIB6) was found to exhibit the same DA pattern on HeLa cells as did the parent strain. This clone contained an 11-kb DNA fragment derived from the largest of the three plasmids, as shown by Southern hybridization. By deletion analysis, a 6.0-kb DNA fragment was shown to be sufficient for expression of the DA phenotype. This insert encoded the production of a 100,000-dalton protein mediating adhesion to HeLa cells.

FILE 'CAPLUS' ENTERED AT 08:34:49 ON 11 MAY 2001
L6 9 S ADHESIN(2W)DIFFUSE(W)ADHERENCE
L7 3 S L6 NOT L2

L7 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:506632 CAPLUS
DOCUMENT NUMBER: 117:106632
TITLE: AIDA-I, the **adhesin** involved in **diffuse adherence** of the diarrheagenic Escherichia coli strain 2787 (O126:H27), is synthesized via a precursor molecule
AUTHOR(S): Benz, Inga; Schmidt, M. Alexander
CORPORATE SOURCE: Zent. Mol. Biol. Heidelberg, Heidelberg, D-6900, Germany
SOURCE: Mol. Microbiol. (1992), 6(11), 1539-46
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The adherence mechanisms of enteropathogenic Escherichia coli (EPEC) to epithelial cells are still not understood. To study the mol. basis of the diffuse adherence (DA) phenotype exhibited by diarrheagenic E. coli expressing classical EPEC serotypes strain 2787 (O126:H27) isolated from a case of infantile diarrhea was investigated. A 6.0 kb plasmid-derived DNA fragment mediates the DA phenotype and encodes the 100 kDa adhesin protein AIDA-I (**adhesin** involved in **diffuse adherence**).
Sequencing of the entire fragment revealed two open reading frames which encoded proteins of 45 kDa and 132 kDa, resp. The 132 kDa protein has been identified as an AIDA-I precursor protein. After cleavage of the signal sequence further processing at the C-terminus of the 132 kDa precursor leads to the mature .apprxeq.100 kDa AIDA-I. While the exact function of the cytoplasmic 45 kDa protein is not known, preliminary evidence indicates that it is necessary for the correct maturation of AIDA-I. The AIDA-I precursor exhibits significant homol. with the virG(icsA) protein of Shigella flexneri which seems to be involved in the intercellular spread of invasive Shigella organisms.

L7 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1990:113026 CAPLUS
DOCUMENT NUMBER: 112:113026
TITLE: Molecular characterization of a fimbrial **adhesin**, F1845, mediating **diffuse adherence** of diarrhea-associated Escherichia coli to HEp-2 cells
AUTHOR(S): Bilge, Sima S.; Clausen, Carla R.; Lau, Wayne; Moseley, Steve L.
CORPORATE SOURCE: Dep. Microbiol., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: J. Bacteriol. (1989), 171(8), 4281-9
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A fimbrial adhesin, designated F1845, was found to be responsible for the diffuse HEp-2 cell adherence of a diarrheal E. coli isolate. The genetic determinant of F1845 was cloned, and the order of the genes necessary for prodn. of F1845 was detd. by maxicell anal. Five polypeptides with apparent sizes of 10, 95, 27, 15.5, and 14.3 kilodaltons (kDa) were found to be encoded in that order by the F1845 determinant. The nucleotide sequence of the 14.3-kDa subunit gene was detd. and found to share extensive homol. in its signal sequence with the gene encoding the structural subunit of the AFA-I hemagglutinin of a uropathogenic E. coli strain but not in the region encoding the mature protein. Southern blot hybridizations indicated that the F1845 determinants are of chromosomal origin. Hybridization studies using a probe from the region encoding the 95-kDa polypeptide indicated that related sequences may be plasmid assocd. in some strains and chromosomal in others. Addnl. hybridization studies of E. coli isolates possessing sequence homol. to the F1845 determinant suggest that the sequences in the 5' region of the F1845 structural subunit gene are more highly conserved than sequences in the 3' region.

L7 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:402085 CAPLUS
DOCUMENT NUMBER: 111:2085
TITLE: Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic Escherichia coli
AUTHOR(S): Benz, Inga; Schmidt, M. Alexander
CORPORATE SOURCE: Zent. Mol. Biol., Univ. Heidelberg, Heidelberg, D-6900, Fed. Rep. Ger.
SOURCE: Infect. Immun. (1989), 57(5), 1506-11
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The adherence of enteropathogenic E. coli (EPEC) to the small bowel mucosa is an important step in the pathogenesis of diarrheal diseases. It has been shown that many EPEC strains adhere to HEp-2 and, esp., HeLa cells in characteristic patterns termed localized adherence (LA) and diffuse adherence (DA). A plasmid-derived DNA fragment encoding a factor specific for LA hybridized only to EPEC strains expressing LA, which demonstrated that LA and DA are mediated by 2 genetically distinct adhesins. EPEC strain 2787 (O127:H27), isolated from a case of infantile diarrhea, exhibited 3 major properties: (i) it showed DA to HeLa cells; (ii) it carried 2 large (.apprx.100 kb) plasmids and 1 small plasmid of .apprx.3 kb; and (iii) no fimbriae could be detected by electron microscopy in organisms grown on agar plates or in liq. cultures. Whole isolated plasmid DNA was partially digested with EcoRI and cloned into the vector pBR322. One recombinant clone (pIB6) exhibited the same DA pattern on HeLa cells as did the parent strain. This clone contained an 11-kb DNA fragment derived from the largest of the 3 plasmids, as shown by Southern hybridization. By deletion anal., a 6.0-kb DNA fragment was shown to be sufficient for expression of the DA phenotype. This insert encoded the prodn. of a 100,000-dalton protein mediating adhesion to HeLa cells.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 08:36:06 ON 11 MAY 2001)

L8 40 S L6
L9 36 S L8 NOT L4
L10 13 DUP REM L9 (23 DUPLICATES REMOVED)

L10 ANSWER 1 OF 13 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001154244 MEDLINE
DOCUMENT NUMBER: 20579451 PubMed ID: 11139209
TITLE: The AIDA autotransporter system is associated with F18 and stx2e in Escherichia coli isolates from pigs diagnosed with edema disease and postweaning diarrhea.
AUTHOR: Niewerth U; Frey A; Voss T; Le Bouguenec C; Baljer G; Franke S; Schmidt M A
CORPORATE SOURCE: Institut fur Infektiologie, Zentrum fur Molekularbiologie der Entzundung, Westfalische Wilhelms-Universitat, Munster, Germany.
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2001 Jan) 8 (1) 143-9.
Journal code: CB7; 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103

ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered PubMed: 20010129
Entered Medline: 20010322

AB Pathogenic Escherichia coli strains are known to cause edema disease (ED) and postweaning diarrhea (PWD) in piglets. Although the exact mechanisms of pathogenicity that lead to ED-PWD remain to be elucidated, E. coli-borne Shiga-like toxin and adhesion-mediating virulence factors such as F18 adhesin or F4 fimbriae are believed to play a central role in ED-PWD. In light of these observations we investigated whether another E. coli adhesin, the plasmid-encoded AIDA (**adhesin** involved in **diffuse adherence**) might also be present in ED-PWD-causing E. coli isolates. For rapid screening for the AIDA system in large numbers of isolates, a multiplex PCR method along with a duplex Western blot procedure was developed. When screening 104 strains obtained from pigs with or without ED-PWD, we observed a high prevalence of the AIDA operon in porcine E. coli isolates, with over 25% of all strains being AIDA positive, and we could demonstrate a significant association of the intact AIDA gene (orfB) with ED-PWD, while defects in orfB were associated with the absence of disease. Although our data hint toward a contribution of AIDA to ED-PWD, further studies will be necessary since the presence of the AIDA genes was also associated with the presence of the Shiga-like toxin and F18 adhesin genes, two reported virulence factors for ED-PWD.

L10 ANSWER 2 OF 13 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000396365 MEDLINE
DOCUMENT NUMBER: 20309702 PubMed ID: 10850987
TITLE: Autodisplay: functional display of active beta-lactamase on the surface of Escherichia coli by the AIDA-I autotransporter.
AUTHOR: Lattemann C T; Maurer J; Gerland E; Meyer T F
CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut fur Biologie, D-72076 Tubingen, Germany.
SOURCE: JOURNAL OF BACTERIOLOGY, (2000 Jul) 182 (13) 3726-33.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000815

AB Members of the protein family of immunoglobulin A1 protease-like autotransporters comprise multidomain precursors consisting of a C-terminal autotransporter domain that promotes the translocation of

N-terminally attached passenger domains across the cell envelopes of gram-negative bacteria. Several autotransporter domains have recently been shown to efficiently promote the export of heterologous passenger domains, opening up an effective tool for surface display of heterologous proteins. Here we report on the autotransporter domain of the Escherichia coli **adhesin** involved in **diffuse adherence** (AIDA-I), which was genetically fused to the C terminus of the periplasmic enzyme beta-lactamase, leading to efficient expression of the fusion protein in E. coli. The beta-lactamase moiety of the fusion protein was presented on the bacterial surface in a stable manner, and the surface-located beta-lactamase was shown to be enzymatically active. Enzymatic activity was completely removed by protease treatment, indicating that surface display of beta-lactamase was almost quantitative. The periplasmic domain of the outer membrane protein OmpA was not affected by externally added proteases, demonstrating that the outer membranes of E. coli cells expressing the beta-lactamase AIDA-I fusion protein remained physiologically intact.

L10 ANSWER 3 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:200006 BIOSIS
DOCUMENT NUMBER: PREV200100200006
TITLE: Bacterial cell surface display of recombinant polypeptides including functional T cell epitopes by the AIDA autotransporter system.
AUTHOR(S): Westendorf, A. M. (1); Lauber, J. (1); Gatzlaff, P. (1); Konieczny, M. P. J.; Schmidt, M. A.; Buer, J. (1); Bruder, D. (1)
CORPORATE SOURCE: (1) Mucosal Immunity Group, German Research Center for Biotechnology, Braunschweig Germany
SOURCE: Immunobiology, (November, 2000) Vol. 203, No. 1-2, pp. 521-522. print.
Meeting Info.: Joint Annual Meeting of the German and Dutch Societies of Immunology Duseldorf, Germany November 29-December 02, 2000
ISSN: 0171-2985.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L10 ANSWER 4 OF 13 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000026814 MEDLINE
DOCUMENT NUMBER: 20026814 PubMed ID: 10559167
TITLE: Characterization of the essential transport function of the AIDA-I autotransporter and evidence supporting structural predictions.
AUTHOR: Maurer J; Jose J; Meyer T F

CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut fur Biologie, D-72076 Tubingen, Germany.
SOURCE: JOURNAL OF BACTERIOLOGY, (1999 Nov) 181 (22) 7014-20. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991213

AB The current model for autodisplay suggests a mechanism that allows a passenger protein to be translocated across the outer membrane by coordinate action of a C-terminal beta-barrel and its preceding linking region. The passenger protein, linker, and beta-barrel are together termed the autotransporter, while the linker and beta-barrel are here referred to as the translocation unit (TU). We characterized the minimal TU necessary for autodisplay with the **adhesin**-involved-in-**diffuse**-**adherence** (AIDA-I) autotransporter. The assumed beta-barrel structure at the C terminus of the AIDA-I autotransporter was studied by constructing a set of seven AIDA-I-cholera toxin B subunit fusion proteins containing various portions of AIDA-I. Surface exposure of the cholera toxin B moiety was assessed by dot blot experiments and trypsin accessibility of the chimeric proteins expressed in Escherichia coli JK321 or UT5600. Export of cholera toxin B strictly depended on a complete predicted beta-barrel region. The absolute necessity for export of a linking region and its influence on expression as an integral part of the TU was also demonstrated. The different electrophoretic mobilities of native and denatured chimeras indicated that the proposed beta-barrel resides within the C-terminal 312 amino acids of AIDA-I. Together these data provide evidence for the predicted beta-barrel structure and support our formerly proposed model of membrane topology of the AIDA-I autotransporter.

L10 ANSWER 5 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1999:323141 BIOSIS
DOCUMENT NUMBER: PREV199900323141
TITLE: Outer membrane integration and transport function of the AIDA autotransporter beta-domain, a heat-modifiable outer membrane protein.
AUTHOR(S): Konieczny, M.P.J. (1); Benz, I. (1); Schmidt, M. A. (1)
CORPORATE SOURCE: (1) Inst. for Infectiology, Ctr. of Molecular Biol. of Inflammation, Univ. of Muenster, Muenster Germany
SOURCE: Abstracts of the General Meeting of the American

Society for Microbiology, (1999) Vol. 99, pp. 39.
Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology
. ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L10 ANSWER 6 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:384418 BIOSIS
DOCUMENT NUMBER: PREV199800384418
TITLE: Interactions of diffuse-adhering Escherichia coli with epithelial cells.
AUTHOR(S): Laarmann, S.; Beinke, C.; Schmidt, M. A.
CORPORATE SOURCE: Inst. Infektiol., Zetrum Molekularbiol., Entzuendung, Westfaelische Wilhelms-Univ. Muenster, 48148 Muenster Germany
SOURCE: European Journal of Cell Biology, (1998) Vol. 75, No. SUPPL. 48, pp. 110.
Meeting Info.: 22nd Annual Meeting of the Deutsche Gesellschaft fuer Zellbiologie (German Society for Cell Biology) Saarbruecken, Germany March 15-19, 1998 German Society for Cell Biology
. ISSN: 0171-9335.
DOCUMENT TYPE: Conference
LANGUAGE: English

L10 ANSWER 7 OF 13 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97158675 MEDLINE
DOCUMENT NUMBER: 97158675 PubMed ID: 9006035
TITLE: Autodisplay: one-component system for efficient surface display and release of soluble recombinant proteins from Escherichia coli.
AUTHOR: Maurer J; Jose J; Meyer T F
CORPORATE SOURCE: Abteilung Infektionsbiologie, Max-Planck-Institut fur Biologie, Tubingen, Germany.
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (3) 794-804.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X65022
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 20000303
Entered Medline: 19970228
AB The immunoglobulin A protease family of secreted proteins are

derived from self-translocating polyprotein precursors which contain C-terminal domains promoting the translocation of the N-terminally attached passenger domains across gram-negative bacterial outer membranes. Computer predictions identified the C-terminal domain of the Escherichia coli **adhesin** involved in **diffuse adherence** (AIDA-I) as a member of the autotransporter family. A model of the beta-barrel structure, proposed to be responsible for outer membrane translocation, served as a basis for the construction of fusion proteins containing heterologous passengers. Autotransporter-mediated surface display (autodisplay) was investigated for the cholera toxin B subunit and the peptide antigen tag PEYFK. Up to 5% of total cellular protein was detectable in the outer membrane as passenger autotransporter fusion protein synthesized under control of the constitutive P(TK) promoter. Efficient presentation of the passenger domains was demonstrated in the outer membrane protease T-deficient (ompT) strain E. coli UT5600 and the ompT dsbA double mutant JK321. Surface exposure was ascertained by enzyme-linked immunosorbent assay, immunofluorescence microscopy, and immunogold electron microscopy using antisera specific for the passenger domains. In strain UT2300 (ompT+), the passenger domains were released from the cell surface by the OmpT protease at a novel specific cleavage site, R / V. Autodisplay represents a useful tool for future protein translocation studies with interesting biotechnological possibilities.

L10 ANSWER 8 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:529547 BIOSIS
DOCUMENT NUMBER: PREV199699251903
TITLE: Processing of the AIDA-I precursor: Removal of AIDA-c and evidence for the outer membrane anchoring as a beta-barrel structure.
AUTHOR(S): Suhr, Martin; Benz, Inga; Schmidt, M. Alexander (1)
CORPORATE SOURCE: (1) Inst. Infektiol., Zentrum Molekularbiol. Entzuendung, Von-Esmarch-Strasse 56, D-48149 Muenster Germany
SOURCE: Molecular Microbiology, (1996) Vol. 22, No. 1, pp. 31-42.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The AIDA-I adhesin known to be responsible for the diffuse adherence (DA) phenotype of the diarrhoeagenic Escherichia coli (DAEC) strain 2787 has been shown previously to be synthesized as a precursor protein and to undergo additional C-terminal processing. Here, the C-terminal processing of the AIDA-I precursor and the outer membrane topology of the cleaved C-terminal fragment, AIDA-c, were investigated. By isolation of the cleaved AIDA-c fragment and N-terminal sequencing, the C-terminal cleavage site was identified

between Ser-846 and Ala-847 thereby indicating a molecular mass of 47.5kDa for AIDA-c. The correct processing to AIDA-I and AIDA-c in OmpT, OmpP and DegP protease-deficient E. coli strains as well as in avirulent salmonellae and shigellae points to an autocatalytic cleavage mechanism. The cleaved AIDA-c was localized in the outer membrane. A leader sequence-AIDA-c fusion was efficiently routed to the outer membrane. Analysis by protease digestion, secondary-structure prediction and modelling, by comparison with structurally related bacterial proteins like the IgA1 protease from neisseria, the vacuolating toxin from Helicobacter pylori, and the VirG protein of Shigella flexneri, strongly indicates that AIDA-c is present in the outer membrane as a beta-barrel structure.

L10 ANSWER 9 OF 13 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 93350332 MEDLINE
DOCUMENT NUMBER: 93350332 PubMed ID: 8347926
TITLE: Diffuse adherence of enteropathogenic Escherichia coli strains--processing of AIDA-I.
AUTHOR: Benz I; Schmidt M A
CORPORATE SOURCE: Zentrum fur Molekulare Biologie Heidelberg (ZMBH), Germany.
SOURCE: ZENTRALBLATT FUR BAKTERIOLOGIE, (1993 Apr) 278 (2-3) 197-208.
Journal code: BD7; 9203851. ISSN: 0934-8840.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931001
Last Updated on STN: 20000303
Entered Medline: 19930916

AB The adherence of pathogenic Escherichia coli to the mucosa of the small intestine is an important step in the development of diarrhoea. To study the molecular basis of the diffuse adherence (DA) pattern of E. coli strains expressing the classical serotypes of enteropathogenic E. coli (EPEC), strain 2787 (O126:H27) was investigated. By expression cloning, a plasmid-derived 6.0 kb DNA fragment was identified which conferred the DA phenotype on recipient K-12 strains. This fragment encoded the 100 kDa **adhesin** involved in **diffuse adherence** (AIDA-I) which by mild heat shock treatment was isolated from the surface of the wild-type and recombinant DA-positive strains. Analysis of the entire DNA fragment revealed two open reading frames coding for proteins of 45 kDa and 132 kDa, respectively. The 132 kDa protein has been identified as the AIDA-I precursor protein which after cleavage of the signal sequence undergoes additional C-terminal processing for maturation to AIDA-I. Though the function

of the cytoplasmic 45 kDa protein is not known, preliminary evidence indicates that authentic expression of the protein is a prerequisite for the correct processing of the 132 kDa precursor to AIDA-I. The AIDA-I precursor exhibits significant homology to the virG (icsA) protein of Shigella flexneri which apparently plays a major role in the events leading to the intercellular spread of invasive Shigella organisms.

L10 ANSWER 10 OF 13 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 92326638 MEDLINE
DOCUMENT NUMBER: 92326638 PubMed ID: 1625582
TITLE: AIDA-I, the **adhesin** involved in **diffuse adherence** of the diarrhoeagenic Escherichia coli strain 2787 (O126:H27), is synthesized via a precursor molecule.
AUTHOR: Benz I; Schmidt M A
CORPORATE SOURCE: Zentrum fur Molekulare Biologie Heidelberg (ZMBH), Germany.
SOURCE: MOLECULAR MICROBIOLOGY, (1992 Jun) 6 (11) 1539-46.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X65022
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920821
Last Updated on STN: 19920821
Entered Medline: 19920813

AB The adherence mechanisms of enteropathogenic Escherichia coli (EPEC) to epithelial cells are still not understood. To study the molecular basis of the diffuse adherence (DA) phenotype exhibited by diarrhoeagenic E. coli expressing classical EPEC serotypes we investigated strain 2787 (O126:H27) isolated from a case of infantile diarrhoea. A 6.0 kb plasmid-derived DNA fragment mediates the DA phenotype and encodes the 100 kDa adhesin protein AIDA-I (**adhesin** involved in **diffuse adherence**).
Sequencing of the entire fragment revealed two open reading frames which encoded proteins of 45 kDa and 132 kDa, respectively. The 132 kDa protein has been identified as an AIDA-I precursor protein. After cleavage of the signal sequence further processing at the C-terminus of the 132 kDa precursor leads to the mature approximately 100 kDa AIDA-I. While the exact function of the cytoplasmic 45 kDa protein is not known, preliminary evidence indicates that it is necessary for the correct maturation of AIDA-I. The AIDA-I precursor exhibits significant homology with the virG(icsA) protein of Shigella flexneri which seems to be involved in the intercellular spread of invasive Shigella organisms.

L10 ANSWER 11 OF 13 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 92104663 MEDLINE
DOCUMENT NUMBER: 92104663 PubMed ID: 1729177
TITLE: Isolation and serologic characterization of AIDA-I, the **adhesin** mediating the **diffuse adherence** phenotype of the diarrhea-associated Escherichia coli strain 2787 (O126:H27).
AUTHOR: Benz I; Schmidt M A
CORPORATE SOURCE: Zentrum fur Molekulare Biologie Heidelberg, Universitat Heidelberg, Germany.
SOURCE: INFECTION AND IMMUNITY, (1992 Jan) 60 (1) 13-8.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920302
Last Updated on STN: 19970203
Entered Medline: 19920212

AB The adherence of diarrhea-associated Escherichia coli to the small-bowel mucosa is an important step in the pathogenesis of diarrheal diseases. In tissue culture systems, diarrhea-associated strains show three distinct patterns of adherence: localized adherence, diffuse adherence (DA), and the recently described aggregative adherence. To study the molecular basis of the DA phenotype, we investigated the diarrhea-associated DA strain 2787 (O126:H27), isolated from a case of infantile diarrhea. The DA phenotype is mediated by a 6.0-kb DNA fragment derived from a 100-kb plasmid harbored by the wild-type strain. This fragment codes for a 100-kDa protein which can be released from the bacterial cell into the supernatant by mild heat treatment. Recombinant DA+ strains as well as the isolated 100-kDa protein were used to engender specific antisera in rabbits. As demonstrated by Western blotting (immunoblotting), the antibodies engendered by the recombinant DA+ strain recognized a 100-kDa protein in the wild-type strain 2787 and in all recombinant strains showing DA. Immunogold electron microscopy localized the 100-kDa protein to the bacterial cell surface. Serologically related proteins of similar size were detected by Western blotting in other DA+ diarrhea-associated strains belonging to enteropathogenic E. coli serotypes. The 100-kDa protein denoted AIDA-I (**adhesin** involved in **diffuse adherence**) binds in a saturable fashion to HeLa cells. AIDA-I-specific immunoglobulin G antibodies--and, to an even greater extent, Fab fragments derived thereof--inhibited bacterial attachment to HeLa cells. This is direct evidence that the

100-kDa protein is the adhesin mediating the DA phenotype of these diarrhea-associated strains and is representative of a group of serologically related proteins in other DA+ strains.

L10 ANSWER 12 OF 13 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 91326955 MEDLINE
DOCUMENT NUMBER: 91326955 PubMed ID: 2101469
TITLE: Diffuse adherence of enteropathogenic Escherichia coli strains.
AUTHOR: Benz I; Schmidt M A
CORPORATE SOURCE: Zentrum fur Molekulare Biologie Heidelberg, Heidelberg, Germany.
SOURCE: RESEARCH IN MICROBIOLOGY, (1990 Sep-Oct) 141 (7-8) 785-6.
Journal code: R6F; 8907468. ISSN: 0923-2508.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19910929
Last Updated on STN: 19970203
Entered Medline: 19910909

AB For the identification and characterization of the factor(s) responsible for the diffuse adherence (DA) pattern of enteropathogenic Escherichia coli strains, E. coli strain 2787 isolated from a case of infantile diarrhoea was employed. A plasmid-derived 11-kb fragment was cloned into pBR322. The recombinant plasmid pIB6 was shown to confer the diffuse adherence phenotype on different E. coli K12 strains as well as pIB4, a plasmid with a 9.2-kb insert. The DNA fragment necessary for the expression of the DA phenotype could be reduced to 6.0 kb. Antiserum obtained against pIB4-encoded proteins recognized a surface-associated protein of about 100 kDa in Western blotting. The isolated 100-kDa protein was found to bind to HeLa cells. The antiserum against C600(pIB4) inhibits adherence of E. coli 2787 and C600(pIB6) to HeLa cells. For this reason, the protein is called **adhesin** involved in **diffuse adherence** (AIDA-I).

L10 ANSWER 13 OF 13 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 89327142 MEDLINE
DOCUMENT NUMBER: 89327142 PubMed ID: 2568985
TITLE: Molecular characterization of a fimbrial **adhesin**, F1845, mediating **diffuse adherence** of diarrhea-associated Escherichia coli to HEp-2 cells.
AUTHOR: Bilge S S; Clausen C R; Lau W; Moseley S L

CORPORATE SOURCE: Department of Microbiology, University of Washington, Seattle 98195.
CONTRACT NUMBER: AI23771 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1989 Aug) 171 (8) 4281-9. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M27725
ENTRY MONTH: 198908
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19970203
Entered Medline: 19890830

AB A fimbrial adhesin, designated F1845, was found to be responsible for the diffuse HEp-2 cell adherence of a diarrheal Escherichia coli isolate. The genetic determinant of F1845 was cloned, and the order of the genes necessary for production of F1845 was determined by maxicell analysis. Five polypeptides with apparent sizes of 10, 95, 27, 15.5, and 14.3 kilodaltons (kDa) were found to be encoded in that order by the F1845 determinant. The nucleotide sequence of the 14.3-kDa subunit gene was determined and found to share extensive homology in its signal sequence with the gene encoding the structural subunit of the AFA-1 hemagglutinin of a uropathogenic E. coli strain (A. Labigne-Roussel, M.A. Schmidt, W. Walz, and S. Falkow, J. Bacteriol. 162:1285-1292, 1985) but not in the region encoding the mature protein. Southern blot hybridizations indicated that the F1845 determinants are of chromosomal origin. Hybridization studies using a probe from the region encoding the 95-kDa polypeptide indicated that related sequences may be plasmid associated in some strains and chromosomal in others. Additional hybridization studies of E. coli isolates possessing sequence homology to the F1845 determinant suggest that the sequences in the 5' region of the F1845 structural subunit gene are more highly conserved than sequences in the 3' region.

FILE 'CAPLUS' ENTERED AT 08:37:46 ON 11 MAY 2001
L11 24 S TRANSPORTER DOMAIN
L12 2 S L1 AND L11
L13 2 S L12 NOT (L2 OR L7)

L13 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:722857 CAPLUS
DOCUMENT NUMBER: 126:27483
TITLE: Molecular analysis of the gat genes from Escherichia coli and of their roles in galactitol transport and metabolism
AUTHOR(S): Nobelmann, Barbara; Lengeler, Joseph W.

CORPORATE SOURCE: Fachbereich Biol./Chem., Univ. Osnabrueck, Osnabrueck, D-49069, Germany
SOURCE: J. Bacteriol. (1996), 178(23), 6790-6795
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In enteric **bacteria**, the hexitol galactitol (Gat) (formerly dulcitol) is taken up through enzyme II (IIGat) of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), and accumulated as galactitol 1-phosphate (Gat1P). The gat genes involved in galactitol metab. have been isolated from the wild-type isolate Escherichia coli EC3132 and cloned on a 7.8-kbp PstI DNA fragment. They comprise six complete open reading frames and one truncated open reading frame in the order gatYZABCDR'. The genes gatABC code for the proteins GatA (150 residues) and GatB (94 residues), which correspond to the hydrophilic domains IIAGat and IIBGat, and GatC, which represents a membrane-bound **transporter domain** IICGat (35 kDa, 427 residues). The three polypeptides together constitute a IIGat of av. size (671 residues). Gene gatD codes for a Gat1P-specific NAD-dependent dehydrogenase (38 kDa, 346 residues), gatZ codes for a protein (42 kDa, 378 residues) of unknown function, and gatY (31 kDa, 286 residues) codes for a D-tagatose-1,6-bisphosphate aldolase with similarity to other known ketose-bisphosphate aldolases. The truncated gatR' gene, whose product shows similarity to the glucitol repressor GutR, closely resembles a gatR gene fragment from E. coli K-12. The gat genes map in both organisms at similar positions, in E. coli K-12, where they are transcribed counterclockwise at precisely 46.7 min or 2,173 to 2,180 kbp. The genes are expressed constitutively in both strains, probably due to a mutation(s) in gatR. Transcription initiation sites for the gatYp and the gatRp promoters were detd. by primer extension anal.

L13 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:623211 CAPLUS
DOCUMENT NUMBER: 121:223211
TITLE: The glucose transporter of Escherichia coli. Overexpression, purification, and characterization of functional domains
AUTHOR(S): Buhr, Andreas; Fluekiger, Karin; Erni, Bernhard
CORPORATE SOURCE: Institute for Biochemistry, University of Bern, Bern, CH-3012, Switz.
SOURCE: J. Biol. Chem. (1994), 269(38), 23437-43
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The glucose transporter of the **bacterial**

phosphotransferase system couples vectorial translocation to phosphorylation of the transported sugar. It consists of a transmembrane subunit (IICBGlc) and a hydrophilic subunit (IIAGlc). The IICBGlc subunit consists of two domains. The NH2-terminal IIC domain (residues 1-386) spans the membrane eight times and contains the substrate binding site. The COOH-terminal hydrophilic IIB domain (residues 391-476) is accessible from the cytoplasmic side of the membrane. It contains the phosphorylation site (Cys421) and together with the IIC domain catalyzes the transfer of phosphoryl groups from the IIAGlc subunit to the transported solute. Starting from a plasmid vector contg. ptsG under an inducible promoter, the IIB and the IIC domains have been subcloned sep., overexpressed in Escherichia coli, and purified by Ni2+ chelate affinity chromatog. Approx. 40 mg of IIBGlc-6H and 4 mg of IICGlc-6H could be purified from 1 L of culture. Cells expressing IIBGlc-6H and IICGlc-6H sep. have a three times longer generation time on glucose minimal medium than cells expressing wild-type IICBGlc. The rate of IIBGlc-6H phosphorylation detd. in a nitrocellulose filter binding assay is indistinguishable from wild-type IICBGlc. The in vitro specific activity of IICGlc-6H in the presence of excess IIBGlc-6H is 2% of the control. IIBGlc-6H also complements the activity of IICBGlc mutant with an inactive IIB domain (C421S) indicating that IIC and IIB are flexibly linked such that a free IIB domain can displace an inactive IIB domain from its contact site on the IIC domain. Based on this work, the secondary structure of the IIBGlc domain has been detd. by isotope-edited NMR spectroscopy (Golic Grdadolnik, et al., 1994).

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 08:38:55 ON 11 MAY 2001)

L14 6 S L12
L15 6 S L14 NOT (L4 OR L9)
L16 3 DUP REM L15 (3 DUPLICATES REMOVED)

L16 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:276722 BIOSIS
DOCUMENT NUMBER: PREV199800276722
TITLE: The glucose transporter of the Escherichia coli phosphotransferase system. Mutant analysis of the invariant arginines, histidines, and domain linker.
AUTHOR(S): Lanz, Regina; Erni, Bernhard (1)
CORPORATE SOURCE: (1) Dep. Chem. Biochem., Univ. Bern, Freiestrasse 3, CH-3012, Bern Switzerland
SOURCE: Journal of Biological Chemistry, (May 15, 1998) Vol. 273, No. 20, pp. 12239-12243.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The glucose transporter of the **bacterial** phosphotransferase system (PTS) consists of a hydrophilic (IIAGlc) and a transmembrane subunit (IICBGle). IICBGlc has two domains (C and B), which are linked by a highly invariant sequence. Transport of glucose by IIC and phosphorylation by IIB are tightly coupled processes. Three motifs that are strongly conserved in 12 homologous PTS transporters, namely two invariant arginines (Arg-424 and Arg-426) adjacent to the phosphorylation site (Cys-421), the invariant interdomain sequence KT-PGRED, and two conserved histidines (His-211 and His-212) in the IIC domain were mutated and the mutant proteins characterized in vivo and in vitro for transport and phosphorylation activity. Replacement of the strongly beta-turn favoring residues Thr and Gly of the linker by alpha-helix favoring Ala results in strong reduction of activity, whereas the substitutions of the other residues have only minor effects. The R424K and R426K mutants can be phosphorylated by IIAGlc but can no longer donate the phosphoryl group to glucose. The H211Q and H212Q mutants continue to phosphorylate glucose at a reduced rate but H212Q can no longer transport glucose. Mixtures of purified R424K/H212Q and R426K/H212Q have 10% of wild-type phosphorylation activity and when coexpressed in Escherichia coli support glucose transport.

L16 ANSWER 2 OF 3 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-480227 [44] WPIDS
DOC. NO. CPI: C1997-152605
TITLE: Presentation of peptide(s) on surface of Gram-negative **bacteria** - via transformation with vector encoding signal peptide, presented peptide and **transporter domain** of **auto-transporter**, producing peptide libraries for epitope mapping.
DERWENT CLASS: B04 D16
INVENTOR(S): JOSE, J; MAURER, J; MEYER, T F
PATENT ASSIGNEE(S): (PLAC) MAX PLANCK GES FOERDERUNG WISSENSCHAFTEN
COUNTRY COUNT: 25
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9735022	A1	19970925	(199744)*	GE	84
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA CN JP KR NZ US					
AU 9651097	A	19971010	(199806)		
EP 886678	A1	19981230	(199905)	GE	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
NZ 304017	A	19990729	(199935)		

CN 1216065 A 19990505 (199936)
AU 714389 B 19991223 (200011)#
JP 2000504928 W 20000425 (200031) 72

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9735022	A1	WO 1996-EP1130	19960315
AU 9651097	A	AU 1996-51097	19960315
		WO 1996-EP1130	19960315
EP 886678	A1	EP 1996-907487	19960315
		WO 1996-EP1130	19960315
NZ 304017	A	NZ 1996-304017	19960315
		WO 1996-EP1130	19960315
CN 1216065	A	CN 1996-180254	19960315
		WO 1996-EP1130	19960315
AU 714389	B	AU 1996-51097	19960315
JP 2000504928	W	WO 1996-EP1130	19960315
		JP 1997-519186	19960315

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9651097	A	Based on	WO 9735022
EP 886678	A1	Based on	WO 9735022
NZ 304017	A	Based on	WO 9735022
AU 714389	B	Previous Publ.	AU 9651097
		Based on	WO 9735022
JP 2000504928	W	Based on	WO 9735022

PRIORITY APPLN. INFO: WO 1996-EP1130 19960315
AN 1997-480227 [44] WPIDS
AB WO 9735022 A UPAB: 19971105
Presentation of (poly)peptides on the surface of Gram-negative **bacteria**, comprises: (a) transforming **bacteria** with a vector that includes (linked to a promoter) a fused nucleic acid sequence (A) consisting of segments encoding a signal peptide, passenger (poly)peptide to be displayed, optional protease recognition site, transmembrane linker and **transporter domain** of an **auto-transporter**; and (b) growing the **bacteria** under conditions where (A) is expressed and the passenger (poly)peptide presented on the surface. The new feature is that the passenger (poly)peptide is heterologous with respect to the transport domain of the **auto-transporter**, which in turn is homologous with respect to the host cell. Also new are: (1) recombinant vector comprising (A); and

(2) gram-negative **bacteria** transformed with the vector.

USE - The method can be used to produce a variegated population of surface-presented (poly)peptides, so that **bacteria** expressing (poly)peptides with particular properties can be identified and simultaneously selected, e.g. for epitope mapping or selection of ligands with the highest affinity for antibodies, major histocompatibility complex (MHC) molecules or other components of the immune system. Selected (poly)peptides can be used diagnostically, e.g. to screen sera or antibody banks, and (poly)peptide expressing cells may be used as live vaccines. They may also be used therapeutically, e.g. when the (poly)peptide is an antibody, to remove or concentrate pollutants, inactivate toxins, prepare and process food, prepare washing compositions and label cells.

ADVANTAGE - Selected **bacteria** can be stored, reproduced and replicated on a large scale as individual clones. (Poly)peptides can now be produced in E. coli, which are easier to manipulate than other **bacteria** previously used, without the problems of compatibility associated with the use of the **transporter domain** of Neisseria gonorrhoeae IgA protease, and the use of a homologous **transporter domain** of an **auto-transporter** improves presentation. Simultaneous selection and identification eliminates the cycles of infection and selection involved in phage display systems, and allows simultaneous amplification of the corresponding gene.

Dwg.0/24

L16 ANSWER 3 OF 3 MEDLINE DUPLICATE 1

ACCESSION NUMBER:	97113438 MEDLINE
DOCUMENT NUMBER:	97113438 PubMed ID: 8955298
TITLE:	Molecular analysis of the gat genes from Escherichia coli and of their roles in galactitol transport and metabolism.
AUTHOR:	Nobelmann B; Lengeler J W
CORPORATE SOURCE:	Universitat Osnabruck, Fachbereich Biologie/Chemie, Federal Republic of Germany.
SOURCE:	JOURNAL OF BACTERIOLOGY, (1996 Dec) 178 (23) 6790-5. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY:	United States Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	199701
ENTRY DATE:	Entered STN: 19970219 Last Updated on STN: 19980206 Entered Medline: 19970128

AB In enteric **bacteria**, the hexitol galactitol (Gat)

(formerly dulcitol) is taken up through enzyme II (II(Gat)) of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), and accumulated as galactitol 1-phosphate (Gat1P). The gat genes involved in galactitol metabolism have been isolated from the wild-type isolate Escherichia coli EC3132 and cloned on a 7.8-kbp PstI DNA fragment. They comprise six complete open reading frames and one truncated open reading frame in the order gatYZABCDR'. The genes gatABC code for the proteins GatA (150 residues) and GatB (94 residues), which correspond to the hydrophilic domains IIA(Gat) and IIB(Gat), and GatC, which represents a membrane-bound **transporter domain** IIC(Gat) (35 kDa, 427 residues). The three polypeptides together constitute a II(Gat) of average size (671 residues). Gene gatD codes for a Gat1P-specific NAD-dependent dehydrogenase (38 kDa, 346 residues), gatZ codes for a protein (42 kDa, 378 residues) of unknown function, and gatY (31 kDa, 286 residues) codes for a D-tagatose-1,6-bisphosphate aldolase with similarity to other known ketose-bisphosphate aldolases. The truncated gatR' gene, whose product shows similarity to the glucitol repressor GutR, closely resembles a gatR gene fragment from E. coli K-12. The gat genes map in both organisms at similar positions, in E. coli K-12, where they are transcribed counterclockwise at precisely 46.7 min or 2,173 to 2,180 kbp. The genes are expressed constitutively in both strains, probably due to a mutation(s) in gatR. Transcription initiation sites for the gatYp and the gatRp promoters were determined by primer extension analysis.

FILE 'HOME' ENTERED AT 08:39:48 ON 11 MAY 2001